REMARKS

THE CLAIM AMENDMENTS

Claims 1, 27, and 28 have been amended to specify that the label probe of step (d)(iii) is an alkaline phosphatase conjugated (AP-conjugated) oligonucleotide probe and that the labeling of step (d)(iv) is carried out with the AP substrate Fast Red. Support for the changes to claims 1, 27, and 28 is found in the specification at *inter alia*, page 19, line 23, to page 20, line 2 (for AP-conjugated probes); page 21, lines 11-14 (for Fast Red); page 22, lines 9-14 (which describes controls that did not have the AP conjugated label probes); page 23, lines 22-24 (further support for the AP-conjugated probes); page 24, lines 1-5 (further support for Fast Red); and page 25, lines 17-20 (further support for Fast Red).

With the change to claim 1 relating to the AP conjugated label probe and the Fast Red AP substrate, claims 16 and 17 have been canceled as reciting redundant subject matter.

In addition to the foregoing, claim 1, which is directed to a method of detecting a nucleic acid analyte comprised of DNA, endogenous genes, and segments thereof, has also been amended to add a recitation at step 1(a)(iii) that any RNA in the sample is digested with RNase. Support for this recitation is found in the specification at page 11, line 28, to page 12, line 5; page 20, lines 4-18 (the recitation directly related to RNase treatment is found at line13); line 22, line 13-16 (which describes the omission of RNase treatment for DNA controls); and page 24, lines 27-30. With the addition of the RNase step at step 1(a)(iii), the subsequent denaturation step was renumbered as step 1(a)(iv).

Lastly, claims 36 and 37 are new. Claim 36 recites that the nucleic acid analyte of claim 27 is located within a subcellular compartment and claim 37 recites that the signal and the nucleic acid analyte are co-localized within a subcellular compartment. Support for claims 36 and 37 is found in the specification at page 3, lines 24-26; page 16, lines 16-21; and Example 3, in particular, page 10-21.

No new matter has been added to the application with the claim amendments made herein.

OBVIOUSNESS REJECTION OVER ANTAO ET AL. IN VIEW OF XU ET AL.

Claims 1, 2-4, 6-23, and 27-33 stand rejected under 35 U.S.C. § 103(a) as obvious over Antao et al. in view of Xu et al. This rejection is respectfully traversed.

At page 6, last paragraph, of the Office Action under reply, the Examiner acknowledges that the instant rejection will be withdrawn upon receipt of the executed Declarations under 37 C.F.R. § 1.131 from all the inventors evidencing that the present invention predates the publication date of the subject matter disclosed in the Antao et al. reference.

The executed Declaration of Daryn Kenny, Ph.D. under 37 C.F.R. § 1.131 was attached to the Amendment filed on February 28, 2005, but the Declarations of the remaining four inventors were not yet

executed and thus, were attached as unexecuted Declarations to the prior Amendment. With this paper, applicants are providing the Office with the executed Declarations under 37 C.F.R. § 1.131 from the remaining four inventors. The Inventor Declarations provide the evidence necessary to overcome this rejection. In view of the submission of the Declarations, applicants respectfully request withdrawal of this rejection.

OBVIOUSNESS REJECTION OVER MORAES ET AL. IN VIEW OF CAO ET AL., NOLTE, SCHAEREN-WIEMERS ET AL., DECIMO, AND XU ET AL.

Claims 1, 3-12, 16, 17, and 20-27 stand rejected under 35 U.S.C. § 103(a) as obvious over Moraes et al. in view of Cao et al., Nolte, Schaeren-Wiemers et al., Decimo, and Xu et al. This rejection is respectfully traversed.

As recited in independent claim 1, the present invention relates to a method for *in situ* detection of DNA, endogenous gene, and segments thereof in a biological sample through the use of *in situ* hybridization and bDNA signal amplification. In steps (d)(iii), claim 1 specifies that the oligonucleotide probes of step(d)(ii) are contacted with an alkaline phosphatase ("AP") conjugated oligonucleotide probe and in step(d)(iv), claim 1 specifies that the probe complex is labeled with the AP substrate Fast Red.

As recited in claim 27, the method of the present invention may be used for identifying the position of a nucleic acid analyte within a cell. In this claim, both RNA and DNA are covered under the scope of the claim and the detection step of the bDNA signal amplification is carried out with the same AP technique specified in claim 1.

At page 11 of the Office Action the Examiner asserts that the detection of a signal would be indicative of the position of the nucleic acid analyte within a cell. Applicants respectfully disagree with the Examiner. As explained at page 24, lines 14-21, of the application, the method of the present invention permits for the detection of a nucleic acid analyte within a particular subcellular compartment of a cell, such as the cytoplasm, nucleus, or an organelle (*see*, page 24, lines 15-16, where it is disclosed that viral mRNA localized predominantly to the cytoplasm whereas viral DNA was restricted to the nucleus). As explained at page 24, lines 16-21, of the instant application, claimed method showed generated positive signals *within the compartment of the cell* in which the target nucleic acid was localized. At page 16, lines 15-17, it is explained that previous *in situ* detection assays were not sensitive enough to locate the subcellular localization of a nucleic acid analyte because signals would diffuse over a relatively large portion of the cell thereby making it impossible to discern the location of the nucleic acid analyte.

Because none of the cited references mention that the procedures disclosed therein may be used for localizing a nucleic acid analyte within a cell, applicants can only surmise that the Examiner is basing the statements made at page 11 of the Office Action as a statement of common knowledge in the art. On this matter, applicants must emphasize that the United States Court of Appeals for the Federal Circuit and its predecessor court the Court of Customs and Patent Appeals have addressed the issue of how common knowledge in the art must be addressed during examination on many occasions, some of which are set forth in the paragraph that follows.

Where an Examiner chooses to take notice of facts beyond the record for the *prima facie* case, those facts must be "capable of such instant and unquestionable demonstration as to defy dispute." *In re Alhert*, 24 F.2d 1099, 1091 (CCPA 1970). It is ***not*** appropriate for an Examiner to take official notice of facts without citing a prior art reference where the facts asserted to be well-known are not capable of *instant and unquestionable demonstration as being well-known. Id.* For example, assertions of technical facts in esoteric technology or specific knowledge of the prior art must always be supported by citation to some reference work recognized as standard in the pertinent art. *Id.*; *see also*, MPEP § 2144.03, 8th ed., Aug. 2001, Rev. Feb. 2003, pp. 2100-131-2100-132; *In re Grose*, 592 F.2d 1161, 1167-1168 (CCPA 1979) ("[W]hen the PTO seeks to rely upon a chemical theory, in establishing a prima facie case of obviousness, it must provide evidentiary support for the existence and meaning of the theory."). More importantly, it has also been established that an Examiner is not at liberty to assert that the state of the art is common knowledge; the state of the art must ***always*** be shown by way of documentary evidence. *See*, MPEP §§ 2144.03, p. 2100-132; *In re Eynde*, 480 F.2d 1364, 1370 (CCPA 1973) ("[W]e reject the notion that judicial or administrative notice may be taken of the state of the art. The facts constituting the state of the art are normally subject to the possibility of rational disagreement among reasonable men and are not amenable to the taking of such notice."). All of these well-established principles of administrative notice were reiterated by the Federal Circuit in the important case, *In re Zurko*, 258 F.3d 1379, 1385 (Fed. Cir. 2001) ("[T]he Board cannot simply reach conclusions based on its own understanding or experience – or on its assessment of what would be basic knowledge or common sense.").

In light of the foregoing guidance provided by the Federal Circuit Court of Appeals, applicants respectfully request that the Examiner provide evidence that the mere identification of a signal would be indicative of the subcellular location of a nucleic acid within a cell, or in the alternative, withdraw claim 27 from the scope of this rejection.

Turning back to the Examiner's substantive rejection, the hypothetical combination of Moraes et al. in view of Cao et al., Nolte, Schaeren-Wiemers et al., Decimo, and Xu et al. does not render the claimed invention obvious for the reasons that follow.

Moraes et al. teaches *in situ* hybridization for the detection of DNA and mRNA. For signal detection of the DNA or mRNA, Moreas et al. uses radiography (*see*, p. 44); accordingly, it follows that Moreas et al. does not teach or suggest the use of AP-conjugated probes and the AP substrate Fast Red for signal detection. Further, as noted by the Examiner on page 8 of the Office Action, Moraes et al. does not teach or suggest the use of bDNA for the detection of the DNA or the RNA. The Examiner cites the combination of Cao et al., Nolte, Schaeren-Wiemers et al., Decimo, and Xu et al. for the missing teaching from Moraes et al. As will be explained below, the secondary references cited by the Examiner do not correct the deficiencies of Moraes et al.

Cao et al. teaches an *in situ* bDNA assay for the detection of mRNA. For the signal amplification, Cao et al. uses AP-conjugated probes and 2-hydroxy-3-naphtoic acid-2′-phenylanilide phosphate ("HNPP") as the AP substrate. Cao et al. does not teach or suggest using the *in situ* bDNA assay for the detection of DNA and further does not teach or suggest using the AP substrate Fast Red for labeling the analyte-target probe-complex.

Nolte teaches the use of bDNA signal amplification for the detection of mRNA from HBV, HCV, and HIV-1 and DNA from HBV. In the last sentence of the reference, Nolte suggests that the bDNA procedure may be used for *in situ* hybridization assays; however, Nolte provides no guidance on how to use the bDNA procedure for *in situ* hybridization assays. When describing the bDNA assay, Nolte describes the known first, second, and third generation bDNA assays and the use of AP probes for detection of the signal (*see*, pages 202-209), but only teaches the use of the AP substrate dioxetane (*see*, page 209) and does not teach or suggest the use of another AP substrate in the labeling step.

The Examiner cites Schaeren-Wiemers et al., Decimo et al., and Xu et al. for teachings relating to the optimization of *in situ* hybridization (*see*, Office Action, pages 9 and 12). The three references each use *in situ* hybridization for detection of mRNA and do not teach or suggest the use of *in situ* hybridization for the detection of DNA. With respect to the visualization of the RNA signal, all three references use different methods of detection: Schaeren-Wiemers uses antibody-labeled probes; Decimo et al. uses radiolabeled probes; and Xu et al. uses hapten-labeled probes.

Neither Schaeren-Wiemers et al., Decimo et al., or Xu et al. express an appreciation for the optimization of an AP substrate to increase sensitivity. In Schaeren-Wiemers et al., the AP-conjugated digoxigenin-labeled ("DIG-labeled") cRNA probes were visualized with X-phosphate, a known chromogenic substrate for the detection of AP also known as BCIP, which stands for 5-bromo-4-chloro-3-indolyl-phosphate (page 432, col. 2). As Decimo et al. only discloses radiography for signal detection (*see*, pages 185-188, which discloses the use of a ^{35}S-labeled probe), it follows that Decimo et al. would not disclose the use of an AP substrate, which they in fact do not. Xu et al. visualized the AP-conjugated

hapten-labeled probes disclosed therein with the chromogenic substrate mix of BCIP and 4-nitroblue tetrazolium chloride ("NBT") for visualization (*see*, pages 87 and 96-98).

Because none of the cited references teach or suggest the use of the AP substrate Fast Red for visualizing AP-conjugated probes, the teachings of Moraes et al. in view of Cao et al., Nolte, Schaeren-Wiemers et al., Decimo et al., and Xu et al. would not lead the ordinary artisan to achieve a successful *in situ* bDNA assay with a sensitivity sufficient to detect DNA or to localize DNA or RNA within a cell. In light of the foregoing, it follows that the invention as recited in claims 1, 3-12, 16, 17, and 20-27 is not rendered obvious by the hypothetical combination of Moraes et al. in view of Cao et al., Nolte, Schaeren-Wiemers et al., Decimo et al., and Xu et al. Because the claimed invention is not rendered obvious by the cited references, applicants respectfully request withdrawal of this rejection.

OBVIOUSNESS REJECTION OVER MORAES ET AL. IN VIEW OF CAO ET AL., NOLTE, SCHAEREN-WIEMERS ET AL., DECIMO, XU ET AL., AND KERN ET AL.

Claims 14 and 15 stand rejected under 35 U.S.C. § 103(a) as obvious over Moraes et al. in view of Cao et al., Nolte, Schaeren-Wiemers et al., Decimo et al., and Xu et al. as applied to claims 1, 3-12, 16, 17, and 20-27 and further in view of Kern et al. This rejection is respectfully traversed.

Claims 14 and 15, which are dependent from claim 1 recite preferred molar concentrations of the preamplifier probes used with the bDNA procedure of the present invention. Because Moraes et al. in view of Cao et al., Nolte, Schaeren-Wiemers et al., Decimo et al., and Xu et al. do not render the invention of claim 1 obvious as explained above, it follows that the additional teachings of Kern et al. will not serve to render claims 14 and 15 obvious. *See, In re Fine*, 837 F.2d 1071 (Fed. Cir. 1988) (If an independent claim is nonobvious under 35 U.S.C. § 103, then any claim depending therefrom is nonobvious.). For the foregoing reason, applicants respectfully request withdrawal of this rejection.

OBVIOUSNESS REJECTION OVER MORAES ET AL. IN VIEW OF CAO ET AL., NOLTE, SCHAEREN-WIEMERS ET AL., DECIMO ET AL., XU ET AL., AND PLUMMER ET AL.

Claims 18, 19 and 28-35 stand rejected under 35 U.S.C. § 103(a) as obvious over Moraes et al. in view of Cao et al., Nolte, Schaeren-Wiemers et al., Decimo et al., and Xu et al. as applied to claims 1, 3-12, 16, 17, and 20-27 and further in view of Plummer et al. This rejection is respectfully traversed.

As recited in independent claim 28, the present invention relates to a method for the detection of 1 to 10 copies of a nucleic acid analyte within a biological sample using an *in situ* bDNA assay wherein the bDNA signal amplification is carried out with an AP conjugated probe the AP substrate Fast Red.

At page 17 of the Office Action, the Examiner acknowledges that the hypothetical combination of Moraes et al. in view of Cao et al., Nolte, Schaeren-Wiemers et al., Decimo et al., and Xu et al. do not teach or suggest an assay sensitivity sufficient to detect 1 to 10 copies of nucleic acid analyte (the Examiner actually states 1 to 2 copies of nucleic acid analyte, which is recited only in claim 19; however, the substance of the Examiner's rejection appears to indicate that this is a typographical error and that the Examiner actually meant 1 to 10 copies of nucleic acid analyte as recited in claims 18 and 28).

Because claims 18 and 19 depend from claim 1 and the combination of Moraes et al. in view of Cao et al., Nolte, Schaeren-Wiemers et al., Decimo et al., and Xu et al. do not render the invention of claim 1 obvious as explained above, it follows that the additional teachings of Plummer et al. will not serve to render claims 18 and 19 obvious. *See, In re Fine.* For this reason, applicants respectfully request withdrawal of this rejection for claims 18 and 19.

With respect to claims 28-35, the Examiner cites Plummer et al. for the missing teaching with respect to heightened sensitivity of *in situ* hybridization

Plummer et al. teaches *in situ* hybridization of low copy number nucleic acids using catalyzed reporter deposition. As noted by the Examiner, Plummer et al. teaches detection of 1-2 copies of HPV DNA in SiHa cells CARD-ISH, which stands for catalyzed reporter deposition *in situ* hybridization (*see*, Office Action, pages 17-18). As explained at page 77, col. 1, of Plummer et al., CARD chemistry uses horseradish peroxidase to catalyze the deposition of reporter-labeled tyramide compounds, such as biotin or fluorescein, to electron-rich moieties present in proteins localized near the enzyme-substrate reaction. Fluorescent-linked tyramides can them be detected using fluorescent microscopy. As is obvious from the foregoing description, Plummer et al. does not teach or suggest using AP-conjugated probes and an AP substrate for visualization of signal; accordingly, it follows that Plummer et al. does not teach or suggest the invention as recited in claim 1.

Notwithstanding the foregoing, applicants take this opportunity to comment on the Examiner's statement at page 18 of the Office Action where the Examiner asserts that because CARD is a signal amplification technique, the ordinary artisan would be led to substitute the bDNA signal amplification technique of Nolte for the CARD technique of Plummer et al. and in so doing would have a reasonable expectation of detecting 1 to 2 copies of HPV DNA. Applicants submit that merely substituting one signal amplification technique for another would not lead the ordinary artisan to have a reasonable expectation of detecting low copy numbers of nucleic acid analytes without some teaching or suggestion that such would be the case. As noted on the first paragraph of Plummer et al., *in situ* hybridization of low copy number DNA and RNA sequences using nonisotopic techniques has not been successful in the past due to low sensitivity. The present invention overcame this deficiency in the art by using an *in situ*

bDNA assay with procedures designed exclusively to improve the sensitivity of *in situ* bDNA such that low copy numbers of both DNA and RNA could be detected; such was accomplished *inter alia* through the use of AP conjugated probes and the AP substrate as set forth throughout the specification and in the Examples (*see*, Example 1, page 21, ll. 8-14; Example 2, page 22, ll. 27-18; and Example 3, page 23, ll. 22 to page 24, line 5; and Example 4, page 25, ll. 9-20). The use of the AP conjugated probes and the AP substrate is now recited in the independent claims.

Because none of the cited references alone or in combination teach or suggest and *in situ* bDNA assay that uses AP conjugated probes and the AP substrate Fast Red to increase sensitivity of the assay, it follows that the invention as recited in claims 28-35 is not rendered obvious by the hypothetical combination of Moraes et al. in view of Cao et al., Nolte, Schaeren-Wiemers et al., Decimo et al., Xu et al. and Plummer et al. For this reasons, applicants respectfully request withdrawal of this rejection for claims 28-35.

OBVIOUSNESS REJECTION OVER PLUMMER ET AL. IN VIEW OF CAO ET AL., NOLTE, DECIMO ET AL., AND XU ET AL.

Claims 28-35 stand rejected under 35 U.S.C. § 103(a) as obvious over Plummer et al. in view of Cao et al., Nolte, Decimo, and Xu et al. This rejection is respectfully traversed.

As recited in claim 28, the method of the present invention may be used for the detection of 1 to 10 copies of a nucleic acid analyte within a biological sample. With this Amendment, claim 28 has been amended to emphasize that the detection step of the bDNA signal amplification is carried out with an AP conjugated probes and the AP substrate Fast Red.

The teachings of Plummer et al. are described above. As stated above, Plummer et al. does not teach or suggest using bDNA for signal amplification and further does not teach or suggest using AP conjugated probes and the AP substrate Fast Red in the CARD assay disclosed therein. The remaining references do not correct the deficiencies of Plummer et al. As previously noted, both Cao et al. and Nolte do not teach or suggest using the AP substrate Fast Red in the *in situ* bDNA assay disclosed therein (Cao et al. teaches the use of HNPP and Nolte teaches the use of dioxetane). Decimo et al. and Xu are cited for specific procedures related to *in situ* hybridization and do not teach or suggest the use of bDNA for signal amplification.

Because the teachings of Plummer et al. in view of Cao et al., Nolte, Decimo, and Xu et al. would not lead the ordinary artisan to an *in situ* bDNA assay with increased sensitivity due to the use of AP conjugated probes and the AP substrate Fast Red, it follows that the hypothetical combination of

Plummer et al. in view of Cao et al., Nolte, Decimo, and Xu et al. does not render the claimed invention obvious. In view of the foregoing, applicants respectfully request withdrawal of this rejection.

CONCLUSION

The foregoing discussion addresses and rebuts each of the Examiner's obviousness rejections. Because the claimed invention is new, useful, and non-obvious, applicants are entitled to a patent grant on the claimed invention. *See, In re Oetiker,* 977 F.2d 1443 (Fed. Cir. 1992) (If examination does not produce a *prima facie* case of unpatentability, then without more, the applicant is entitled to a grant of the patent). For the foregoing reasons, applicants respectfully request withdrawal of all outstanding rejections for this application and passage of this application to allowance.

If the Examiner has any questions regarding this Amendment that may be addressed by way of a telephone call or e-mail correspondence, she is encouraged to contact the undersigned at 650-251-7713 or canaan@reedpatent.com.

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